

### REMARKS

This is in response to the Final Office Action dated September 28, 2009. A Request for Continued Examination is submitted contemporaneously herewith.

The withdrawn rejections enumerated on pages 2 and 3 of the Action are noted. The asserted objections on pages 4-5 of the Action have been overcome by the cancellation herewith of claims 29-31, 33 and 53. Moreover, as discussed below, main claim 22 has now been amended to specify that the promoter is a seed-specific promoter, the specific use of which accomplishes new and unexpected results as corroborated in the Declaration of Valerie Frankard submitted herewith.

Claim 32 has been amended as above. Any purported deficiency in claim 32 for reciting "under stringent conditions" has been overcome by extracting the stringent condition parameters from the specification, published paragraph 43, and reciting them in the claim. Substantively, the ability of one skilled in the art to determine hybridizing sequences to SEQ ID NO 1 should be assessed in light of the specification and its specific published paragraph 43 reference to Sambrook (2001) Molecular Cloning: a laboratory manual, 3<sup>rd</sup> Edition Cold Spring Harbor Laboratory Press, CSH, New York, not the much earlier Maniatis et al. 1982 reference cited by the Examiner. The specification citation to Sambrook et al. details throughout the lengthy specification published paragraph 43 how one skilled in the art knows how to make a sequence that hybridizes as set forth in amended claim 32. Moreover, the EST example on pages 30-32 of the United States Patent and Trademark Office Written Description Guidelines (found at this writing at <http://www.uspto.gov/web/offices/pac/writtendesc.pdf>) is nonanalogous, because in that EST example the partial cDNA at issue was not even disclosed as to whether it crosses an exon/intron splice site, whereas the present SEQ ID NO. 1 represents a well known sequence underlying a well-known NHX protein and those skilled in the art are able to understand paragraph 43 of the specification to identify and practice such a hybridizing sequence in a minimal number of tries. Amended claim 32 now satisfies all 37 C.F.R. Sections 112 first and second paragraph requirements.

The asserted prima facie obviousness rejections over Fukuda et al., Wu et al. and Chan et al., all of record, are overcome by the rebuttal evidence provided in the accompanying Declaration of Valerie Frankard. As explained further below, the Declaration provides

comparative evidence to corroborate that the claimed invention, practiced with a seed-specific promoter, gives new and unexpected results in contrast to the same method practiced with either a root- or shoot-specific promoter. The prior art generally provides combinatorial teachings that sequences such as that claimed can be used with any sort of promoter, and the new and unexpected results possible with the seed-specific promoter, and in particular as claimed, are only apparent to one skilled in the art after consultation is made to the present specification. The details underlying this position of Applicant are set forth below.

EP 1,143,002 to Fukuda et al. discloses an antiporter gene pertinent to NHX1 but does not suggest that any sort of tissue-specific promoter should be used with it in any context. The only promoter identified at all in EP 1,143,002 is the cauliflower mosaic virus 35S promoter discussed in Example 3, and cauliflower mosaic virus 35S (CMV 35S) is widely understood to be a constitutive promoter (see the attached November 6, 2009 information from <http://www.patentlens.net/daisy/promoters/242/g1/250.htm>). In fact, the aforementioned cited reference describes CMV 35S as “a very strong constitutive promoter, causing high levels of gene expression in dicot plants...” and “[h]owever, it is less effective in monocots, especially cereals.” One would therefore not learn which if any promoters to use in monocots from Fukuda et al., and Fukuda et al. EP 1,143,002 does not provide any insight as to any other promoter to use because it mentions only one. However, the counterpart U.S. Patent to the Fukuda et al. reference of record, namely, U.S. Patent No. 6,861,574, which claims priority to the common PCT/JP99/07224 as does EP 1,143,002, discloses a second promoter in Example 4 which is not included in the EP 1,143,002 **and this second promoter, GAP promoter, is also a constitutive promoter** (see, for example, <http://www.ncbi.nlm.nih.gov/pubmed/18781398> (copy attached) which shows in the abstract that GAP is a constitutive promoter). The Examiner will of course note that the GAP protein (Glyceraldehyde-3-phosphate dehydrogenase) is a housekeeping gene that is involved in glycolysis, one of the basic pathways in eukaryotic cells for generating energy in a cell. One skilled in the art, therefore, consulting the two Fukuda et al. specifications, would learn only that the antiporter gene is susceptible of manipulation with a constitutive promoter, nothing more. It is anticipated that the Examiner will make U.S. 6,861,574 of record inasmuch as it is cited in an Information Disclosure Statement filed of even date.

Chan et al., of record, disclose in the abstract that “the rice alpha-amylase promoter functions in all cell types of the mature leaves, stems, sheaths and roots, but not in the very young leaves.” Thus Chan et al. admit by deliberate omission, right in the abstract, that in the context of the transgenic rice technology of the paper, no seed-specific promoter is disclosed at all. Even more importantly, as the Examiner states in the September 28, 2009 Office Action page 17, “[i]t is further maintained that at the time the invention was made, it would have been *prima facie* obvious to one of ordinary skill in the art to modify the method of making a transgenic monocotyledonous plant (rice) as taught by Fukuda et al., **to substitute the CaMV 35S promoter with any other constitutive promoter, including the nos promoter of Chan et al., to arrive at the instantly claimed invention with reasonable expectation of success**” [emphasis added]. Thus the Examiner has already recognized of record that the claimed invention, particularly limited to seed-specific promoters, is not taught when Fukuda et al. and Chan et al. are considered together.

That Wu et al. are directed to a seed-specific promoter is apparent from the title, “Promoters of Rice Seed Storage Protein Genes Direct Endosperm-Specific Gene Expression.” From a patent examining standpoint, one finds what one is looking for, and certainly Wu et al. discusses endosperm-specific expression. However, there is nothing in Wu et al. to suggest that a seed specific promoter, in particular, should be combined with the NHX gene in the present method to increase yield/biomass or to modify plant architecture—not to impart salt tolerance. Wu et al., Fukuda et al. and Chan et al. considered in the aggregate suggest that constitutive promoters should be used with NHX and seed-specific promoters should be used when endosperm-specific gene expression is desired—the latter of which is NOT the improved yield/biomass or modified plant architecture of the present claim 22.

The three cited references already of record are best understood in light of a newly identified prior art reference, namely, U.S. Patent No. 6,936,750, a copy of which is cited on the enclosed Information Disclosure Statement. This newly-cited patent reference addresses sodium/hydrogen transporter genes and furthermore lists many purportedly suitable promoters, such as “a super promoter, a 35S promoter of cauliflower mosaic virus, a drought-inducible promoter, an ABA-inducible promoter, a heat shock-inducible promoter, a salt-inducible promoter, a copper-inducible promoter, a steroid-inducible promoter and a tissue-specific

Application No. 10/559,097  
Amendment dated January 28, 2010  
In Reply to Office Action of September 28, 2010  
Attorney Docket No. 4559-053584

promoter” but nowhere mentions any seed specific promoter(s). This patent makes clear that as to an NHX gene, many kinds of promoters are contemplated **but nowhere in this reference are seed-specific promoters even mentioned, let alone highlighted for their ability to achieve new and unexpected results in improved yield/biomass or modified plant architecture.** The aggregated prior art thus clearly cannot point to the new and unexpected results attributable to the claimed invention when practiced, specifically among its recited method steps, with a seed-specific promoter to achieve modified plant architecture or increased yield/biomass, particularly in view of the corroborating data in the enclosed Declaration of Valerie Frankard.

The accompanying Declaration of Valerie Frankard is largely self-explanatory and tightly confirms the above assertions. Paragraph 2 presents data for seed-, root- and shoot-specific promoters (with data additionally attached and referred to) and after presenting and explaining further data in Paragraphs 3-6, Dr. Frankard reaches the conclusion that the claimed invention, reciting a seed-specific promoter, “gives new and unexpected results over the same method practiced with a root- or shoot-specific promoter.” The asserted prima facie obviousness rejections may all be seen to have been rebutted, and overcome, by the data and conclusions inherent in the Declaration submitted herewith and in view of the inherent inability of the prior art to point to seed-specific promoters, in particular, as giving new and unexpected results in the claimed context.

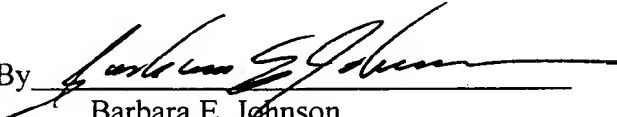
Application No. 10/559,097  
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CONCLUSION

Claims 22, 25, 28, 32 and 52 are now in condition for allowance. Should any issue arise which can be resolved by telephone, the undersigned respectfully requests a telephone call to her direct dial number, 412-281-3350.

Respectfully submitted,

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Performing your original search, **GAP promoter**, in PubMed will retrieve **650** records.

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Mol Biol Rep. 2009 Jul;36(6):1611-9. Epub 2008 Sep 10.

### Recent advances on the GAP promoter derived expression system of *Pichia pastoris*.

Zhang AL, Luo JX, Zhang TY, Pan YW, Tan YH, Fu CY, Tu FZ.

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*Pichia pastoris* is an efficient host for the expression and secretion of heterologous proteins and the most important feature of *P. pastoris* is the existence of a strong and tightly regulated promoter from the alcohol oxidase I (AOX1) gene. The AOX1 promoter (pAOX1) has been used to express foreign genes and to produce a variety of recombinant proteins in *P. pastoris*. However, some efforts have been made to develop new alternative promoters to pAOX1 to avoid the use of methanol. The glyceraldehyde-3-phosphate dehydrogenase promoter (pGAP) has been used for constitutive expression of many heterologous proteins. The pGAP-based expression system is more suitable for large-scale production because the hazard and cost associated with the storage and delivery of large volume of methanol are eliminated. Some important developments and features of this expression system will be summarized in this review.

PMID: 18781398 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

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## The CaMV 35S promoter

Analysis on the CaMV 35 promoter is divided into a discussion of:

- the promoter itself
- sequences identified in patents as "35S enhancer regions"
- the "minimal" promoter

### The promoter itself

#### Scientific aspects

At the beginning of the 1980s, Chua and collaborators at the Rockefeller University isolated the promoter responsible for the transcription of the whole genome of a Cauliflower mosaic virus (CaMV) infecting turnips. The promoter was named **CaMV 35S promoter** ("35S promoter") because the coefficient of sedimentation of the viral transcript whose expression is naturally driven by this promoter is 35S. It is one of the most widely used, general-purpose constitutive promoters.

The 35S promoter is a very strong constitutive promoter, causing high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors.

#### Nucleotide sequence of the CaMV 35S promoter (-343 to +1)

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-343                                     -300
5' tgagactttt caacaaaggg taatatccgg aaacctcctc ggattccatt
   gccagctat ctgtcacttt attgtgaaga tagtggaata ggaaggtggc
   tcctacaaat gccatcattg cgataaagga aaggccatcg ttgaagatgc
   ctctgccgac agtgggccca aagatggacc cccacccccc gaggagcatc
   gtggaaaaag aagacgttcc aaccacgtct tcaaagcaag tggattgatg
   tgatatctcc actgacgtaa gggatgacgc acaatccccc tacccttcgc
   aagacccttc ctctatataa ggaagtccat ttcatttgga gagga 3'
                                     +1

TATA box tatataa
CAAT sequences

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The promoter responsible for the transcription of another part of the genome of CaMV, the CaMV 19S promoter, is also used as a constitutive promoter, but is not as widely used as the 35S promoter.

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